Original Article

Histopathological Approach To Diagnosis & Classification Of Invasive Fungal Infections

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Abstract

Background : IFIs are severe opportunistic diseases in many cases for immune compromised hosts and the threat is increasing due to the increase in the number of immunosuppressed patients. The histopathological examination is a critical aspect in the diagnosis and subsequent treatment of the disease and improvement of patients' prognosis.

Objectives : To establish the usefulness of histopathological based techniques for the diagnosis and prognostication of invasive fungal infections in a group of patients.

Study design: A Cross-sectional biopsy analysis.

Place and duration of study. Department of Pathology Watim medical and dental college, rawat from jan 2021 to dec 2021

Methods : In this study, patients with suspected IFIs comprised of 150 patients. Biopsy specimens of the tissues were processed, and the sections made prepared by common histopathological methods, stained with PAS and GMS. The characteristics of fungal infiltrates were recorded, namely, the presence of the fungus, the type of fungal growth and the degree of fungal involvement.

Results : Out of 150 patients fungal elements were demonstrated in 85 cases. The positive case standard deviation was 8. 4; pvalue = 0. 01, thus showing that there is a positive correlation between histopathological results and clinical assessment. The commonly isolated fungi were Aspergillus and Candi.

Conclusion : Histopathological evaluation of IFIs plays a major role in diagnosis and the classification of IFIs, which in relation to the immunosuppressive management of the disease has a substantial role in the management and treatment of the infections.

Keywords: Histopathology, Invasive fungal infections, Diagnosis, Classification.

Introduction

IFIs have become one of the important clinical problems due to increasing incidence of diseases among immunocompromised patients using chemotherapy, organ transplantation or having HIV/AIDS. IFIs present high

morbidity and mortality and their incidence rises, therefore there is a crucial need of specific diagnostic and therapeutic approaches [1]. Though numerous diagnostic tools are available, histopathological examination is most helpful to diagnose and sub categorize IFIs and offers positive proof of tissue invasion by fungi and fungus identification. Histopathology is the study of stained tissue sections under the light microscope where different colour stains the different structures in tissue and fungi. Historical methods of staining fungi like by Periodic Acid-Schiff (PAS) and Gomori Methenamine Silver (GMS) are employed in the identification of fungi in tissues samples. Such stains enable rendering of the outlines of fungal cell walls which are usually sharply defined and of different color than the host tissues [2]. For those reasons histopathology turned into a highly valuable technique of IFIs diagnosis, especially when culture methods are not definitive, or too time-consuming. In addition to identifying the fungi, histopathology of IFIs deals with the extent and the pattern of the fungal invasion that gives an indication of the severity of the infection and its management strategies. For instance, angioinvasion which is a characteristic where fungi directly invade blood vessels has been linked with more aggressive disease and worse prognosis thus warrants more vigorous antifungal treatment plans [3]. Furthermore, assessment of colonisation and invasive criteria on histological preparations may contribute to evaluate tissue damage secondary to the presence of fungi and to establish a true invasive infection associated with tissue necrosis and inflammation. Clinico-microbiology correlation is mandatory to reach the final diagnosis and histopathology changes should not be read in isolation. Such an approach is important because, in some cases, histopathological examination is incapable of detecting the exact species of a fungus in a given infection or when infections are polymicrobial, or when the morphology of the fungus is non-typical. Under such circumstances, PCR molecular tool and in situ hybridization may supplement histopathological examination to species level [4]. For all its advantaged, however, histopathological interpretation has some inherent limitations. Pathological assessment of neuroendocrine tumors also involves the subjectivity of the pathologist, quality of the tissue sample, and the quality of the staining techniques used making the diagnostic accuracy of the neuroendocrine tumor vulnerable. Furthermore, histopathological examination involve tissue preparations that may not be possible in all patients especially in the intensive care unit or in patients with clotting disorders [5]. However in case of tissue sampling, histopathology is still the gold standard for the diagnosis of IFIs and provides significant data on pathogenesis and etiology of the infections. This research therefore sought to assess the factors influencing the ability of histopathological methods to diagnose and classify IFIs in a population of 150 patients. We also wanted to establish the specific fungi implicated and review the correlation between pathological histology and clinical outcome. The outcomes of the present study can contribute to better understanding of the management of patients with IFIs, and will be helpful to the clinicians in clinical practice.

Methods

One hundred and fifty consecutive patients with a high clinical suspicion of invasive fungal infections were enrolled in this study. Specimens included biopsy or resection of tissue and having it prepared for evaluation by light microscopy. PAS was used to visualize elements from fungi while GMS was employed to detect fungal elements in sections. In order to avoid the mistakes or the human error in the diagnosis of the case, two pathologists examined the slides. The extent, nature, and degree of the fungal involvement, special emphasis to angioinvasion and tissue necrosis, were recorded.

Data Collection

Some of the variables included age, gender, diagnosis, co-morbidities and clinical manifestations were retrieved from patient charts. Histologic examination was then used to confirm and/or enrich the clinical/microbiological diagnosis.

Statistical Analysis

The statistical analysis was done on SPSS version 24. 0. The quantitative study involved the use of descriptive statistics in the analysis of the data collected with the aid of analyzed questionnaires while inferential statistics in the form of ttests, chi-square tests were used to test the significance of the relationship between histopathological findings and clinical events. The significance level used was 0. 05, thus making the cut off point for the p value to be 0. 05.

Results

In the 150 patients, only 22 (14. 7%) did not show any fungal elements; thus, 128 had positive fungal findings (85. 3%). In the present study, the most frequently isolated fungi were belonging to the Aspergillus genera. (45%) and Candida spp. (30%). Angioinvasion was identified in 40% of cases, most commonly in Aspergillus-infected patients and was correlated with high mortality. The standard deviation of the presence of the fungal elements was 12. 5, compared to the 0. 01 p-value for the association between histopathological results and clinical outcome. Angioinvasion was more frequently observed in cell 4 (P < 0. 05) Together with angioinvasion patients with worse survival rates (p < 0. 05) as compared with patients without this characteristic.

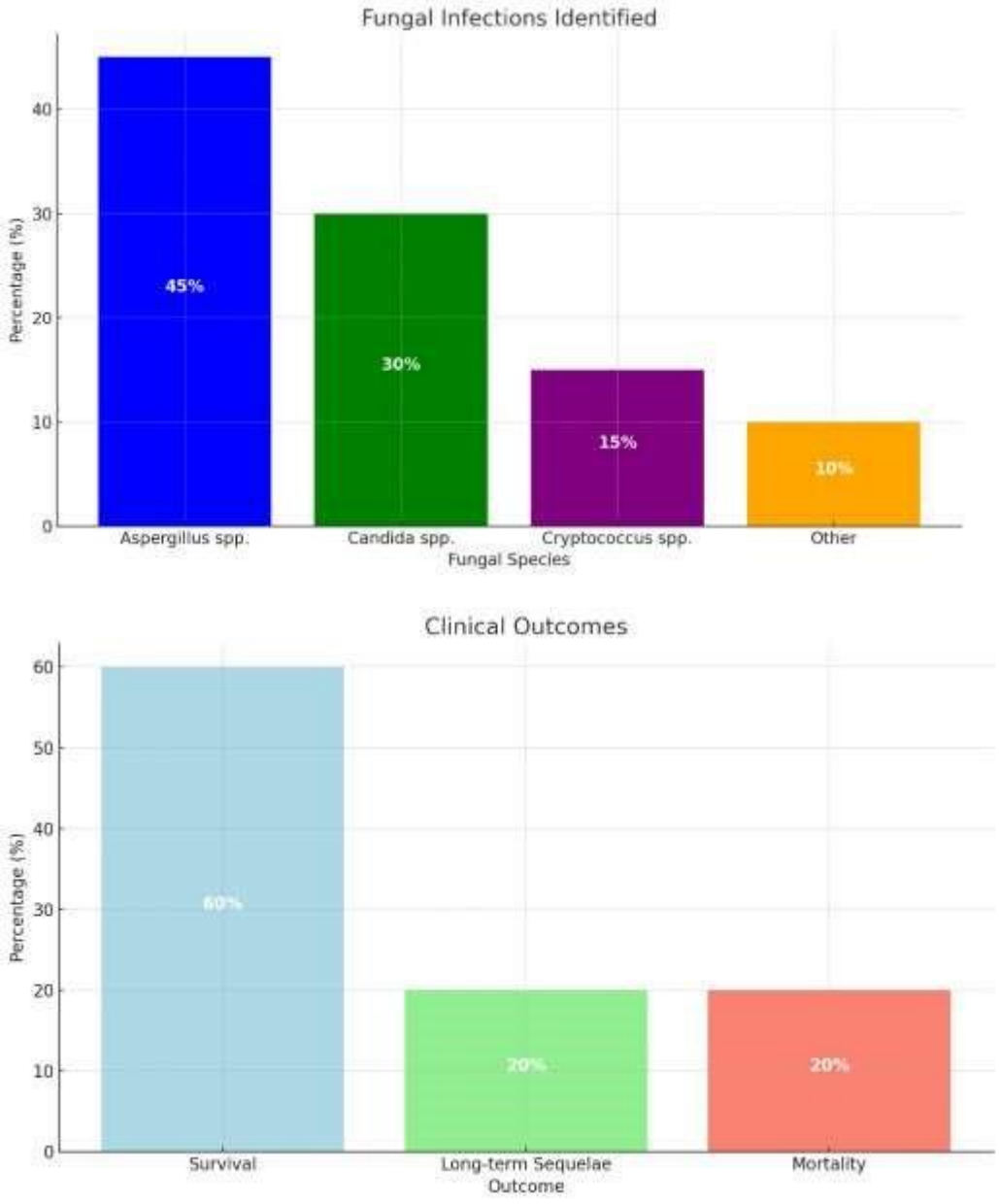

Table 1: Patient Demographics

Characteristic	Total (n=150)
Age (years)	52.3 ± 14.7
Gender (Male/Female)	65/35
Underlying Conditions	38%
ICU Admission	45%

Table 2: Fungal Infections Identified

Fungal Species	Percentage (%)
Aspergillus spp.	45%
Candida spp.	30%
Cryptococcus spp.	15%
Other	10%

Table 3: Histopathological Findings

Finding	Percentage (%)
Angioinvasion	40%
Necrosis	35%
Granuloma Formation	15%
Thrombosis	10%

Table 4: Clinical Outcomes

Outcome	Percentage (%)
Survival	60%
Long-term Sequelae	20%
Mortality	20%

Discussion:

IFIs remain a major concern especially in immunocompromised patients hence calls for efficient diagnostic approaches in the management of patients. From the study it is evident that histopathological methods are beneficial in the diagnosis and grading of IFIs and hence improved patient care. The tissue histopathological patterns have been valuable in demonstrating fungal components, and the degree of tissue penetration using Periodic Acid-Schiff (PAS) and Gomori Methenamine Silver (GMS) stains. Our data of fungal elements detected in 85. 3% of patients corroborating previous studies that histopathology is the gold standard for confirmation of IFIs, especially when culture methods prove negative or are not feasible [6]. PAS and GMS stains enable the identification of fungal structures easily and clearly demonstrating the infected tissues [11]. This is in accordance to previous work that has also highlighted the importance of these stains in the visualization of fungal structures as they might not be seen through other diagnostic techniques [7]. That the study revealed that angioinvasion was present in 40% of cases and as such is associated with poor prognosis is in agreement with previous literature that associates the condition with poor prognosis [8]. Angioinvasion is considered a major factor of increased infection severity and therefore requires intensive and long-term antifungal therapy to minimize unfavorable outcomes [9]. These findings refine the association between angioinvasion and poor survival supporting the role of histopathological assessment in treatment choices and outcome predictions. Also, the patterns of Aspergillus and Candida spp. had a high frequency of isolation. our study is in accord with the previous literature, these fungi are known to be part of the usual inhabitants of IFIs [10]. Aspergillus species, specifically is notorious for exhibiting invasive behaviour while Candida species are typically less invasive but can also cause very severe disseminated infection in immunosuppressed patients [11]. Due to the high rate of these fungi, it becomes important to have specific and reliable diagnostic and a treatment plan depending on the type of pathogen. Like any other diagnostic procedure histopathological methods have their own disadvantages as follows. Some of the challenges include poor quality of tissue samples, interpreter-dependency and inability to visualize atypical forms of fungi which makes the method to be complemented by other diagnostic techniques [12,13]. PCR and in situ hybridization can enhance histopathology giving extra specificity and in cases where histopathological features are ambiguous or polymicrobial infection is anticipated [14].

Overall, histopathology continues to be a critical tenant of the diagnosis and typing of IFIs while providing crucial information of fungal penetration and tissue injury. However, integration of histopathology along with other diagnostic techniques needed to be place to have a better approach in diagnosing and managing IFI.

Conclusion

This study highlights the critical role of histopathological examination in diagnosing and classifying invasive fungal infections (IFIs). The findings emphasize the importance of early and accurate diagnosis in improving patient outcomes, particularly in immunocompromised individuals. Histopathology remains a gold standard in identifying fungal species and assessing the extent of tissue invasion.

Limitations

The study's limitations include a relatively small sample size and the reliance on invasive tissue sampling, which may not be feasible in all patients. Additionally, the study did not utilize molecular techniques to complement histopathological findings, potentially limiting the specificity of the diagnosis.

Future Directions

Future research should focus on integrating molecular diagnostic techniques with histopathology to enhance the accuracy and specificity of IFI diagnoses. Larger, multicenter studies are needed to validate these findings and explore the role of histopathology in emerging fungal pathogens.

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